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## (54) Improvements in or relating to sample slides

(57) A sample slide (10) is prepared for use for, e.g., immunofluorescence assay, by a method comprising forming permanent markings (12, 14) on a slide so as to divide the sample-bearing surface thereof into an array of discreet areas; cleaning at least the sample-bearing surface of the slide; applying masking medium such as varnish to the sample-bearing surface of the slide to cover the marked,

discreet areas; treating at least the sample-bearing surface of the slide with a hydrophobic reagent; cleaning and polishing the sample-bearing surface of the slide; and removing the masking medium. The resulting slide has a hydrophobic covering on the sample-bearing surface thereof defining an array of discreet uncovered areas (where the masking medium was) separated by the hydrophobic covering. In use, small quantities of samples may be applied to the uncovered areas. In practice it is found that the covered areas of the slide are genuinely hydrophobic, thus eliminating or at least substantially reducing the risk of samples drying out, which has hitherto been a problem.

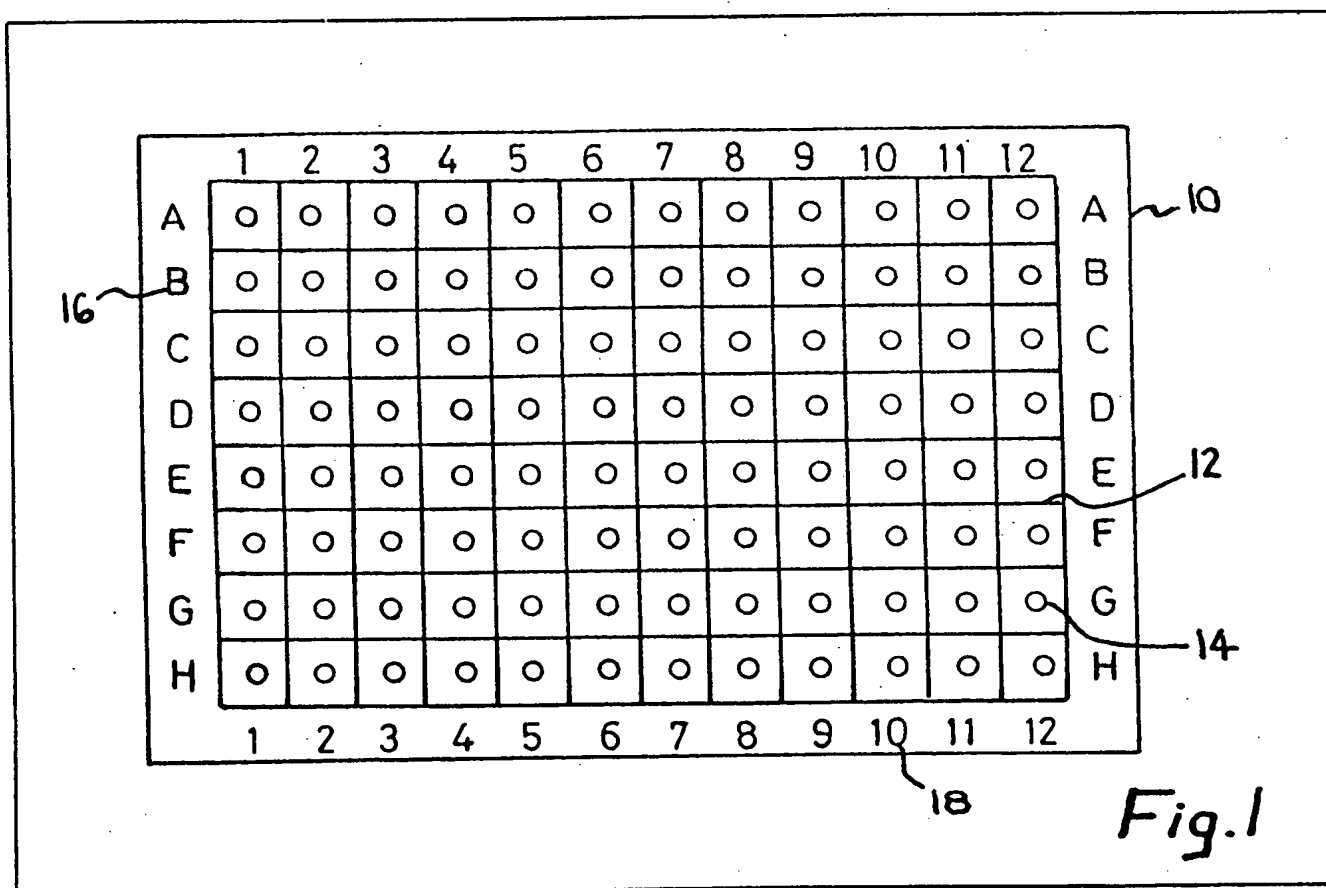


Fig. 1

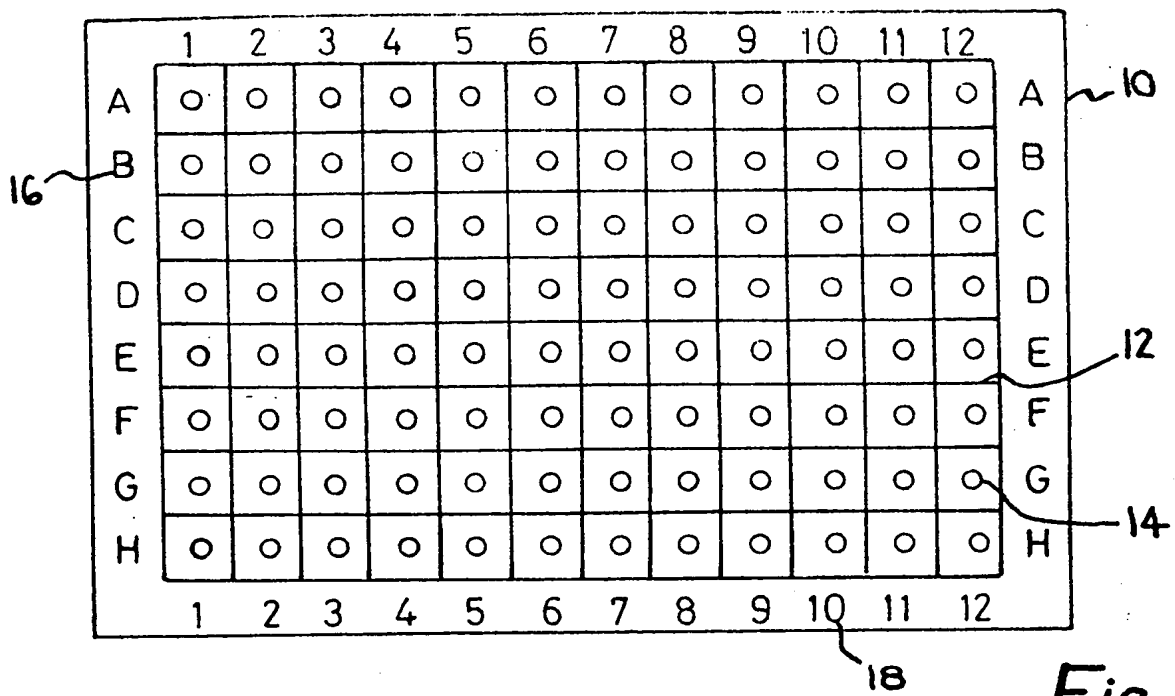


Fig. 1

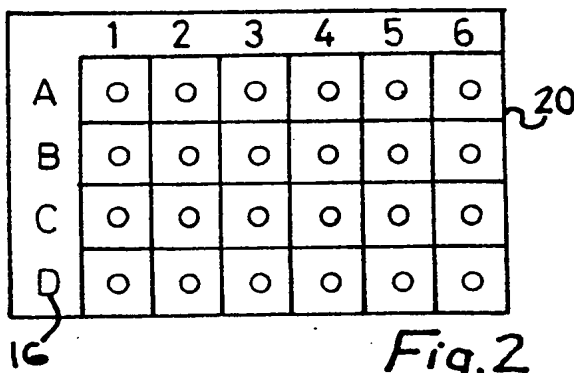


Fig. 2

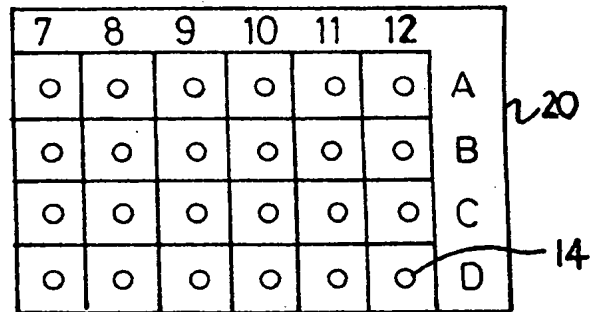


Fig. 3

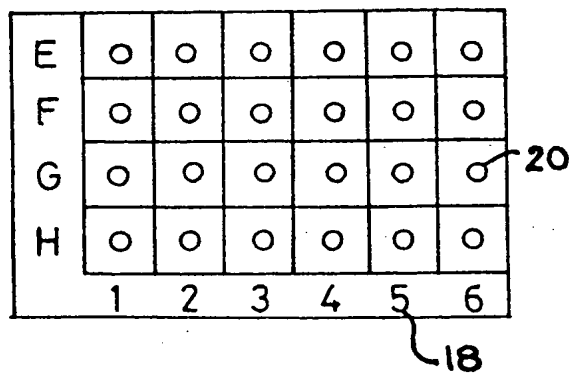


Fig. 4

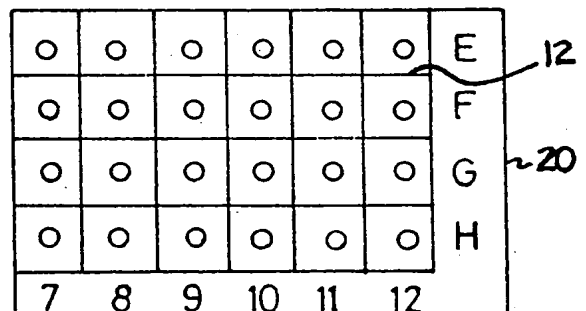


Fig. 5

**SPECIFICATION****Improvements in or relating to sample slides****Field of Invention**

- 5 This invention relates to sample slides and concerns a method of preparing slides suitable for use for, for example, immunofluorescence assay, and also a slide prepared by the method of the invention.

**Background to the Invention**

- 10 When carrying out diagnosis, e.g. by immunofluorescence assay, a small quantity of, e.g. one drop, of a sample is generally located on a slide, and in order to avoid contamination between different samples traditional practice has been to employ individual slides for each sample, with say one drop of sample being applied per slide.

- 15 In an attempt to improve this arrangement multi-sample slides have been developed in which a Teflon (RTM) coating is bonded to a slide so as to define discreet circular uncovered areas of the slide to which different samples can be applied. In theory this technique enables say eight different samples to be applied per slide. However, problems arise in practice.

- 20 1) Although Teflon in theory is hydrophobic it tends to wet to a certain degree. This leads to problems with samples drying out.

- 25 2) Wetting of the Teflon barriers leads to possible bridging and contamination between adjacent samples.

- 30 3) Teflon is attacked by acetone/methanol fixative which is required for use in certain techniques.

- 35 The present invention seeks to provide a multi-sample slide in which the above disadvantages are eliminated or at least significantly reduced.

**The Invention**

- 40 According to the present invention there is provided a method of preparing a sample slide, comprising:

- 1) forming permanent markings on a slide so as to divide the sample-bearing surface thereof into an array of discreet areas;

- 45 2) cleaning at least the sample-bearing surface of the slide;

- 3) applying masking medium to the sample-bearing surface of the slide to cover the marked, discreet areas;

- 50 4) treating at least the sample-bearing surface of the slide with a hydrophobic reagent;

- 5) cleaning and polishing the sample-bearing surface of the slide; and

- 6) removing the masking medium.

- 55 The resulting slide has a hydrophobic covering on the sample-bearing surface thereof defining an array of discreet uncovered areas (where the masking medium was) separated by the hydrophobic covering.

- 60 In use, small quantities of samples may be applied to the uncovered areas. By making the areas small, very small sample and reagent volumes are required, typically in the range 10  $\mu$ l

to 25  $\mu$ l, and possibly even as low as 5  $\mu$ l if the slide is pre-wetted. Minimal use of valuable reagents is thus facilitated.

- 65 In practice it is found that the covered areas of the slide are genuinely hydrophobic, thus eliminating or at least substantially reducing the risk of samples drying out, which has hitherto been a problem.

- 70 The markings are conveniently in the form of circles in a rectilinear array. A rectilinear grid of lines is desirably also marked on the slide, with a respective circle located in each area of the grid, to assist in identification of the locations of the circular areas. Reference markings may conveniently be provided at the edges of the grid, e.g. in the form of letters and numbers, to assist in identification of individual areas.

- 80 The markings may conveniently be produced by a printing process. Alternatively, a transfer technique may be employed, with the slide being heated to bake the transfer permanently on to the slide. In both of these cases, the markings are preferably made on the sample-bearing surface of the slide so that the markings will be at the same general vertical level as the samples, thus facilitating focusing when examining the samples under a microscope. As an alternative possibility the markings may be produced by an etching technique. In this case the markings are preferably made on the opposed surface of the slide to eliminate the possibility of samples bridging the etched regions.

- 95 The masking medium may be any suitable material which does not react adversely with the other materials involved and which can be removed in appropriate manner. For example, the masking medium conveniently comprises varnish, e.g. nail varnish, such material being suitable inert, easy to apply and to remove.

- 100 The hydrophobic reagent conveniently comprises a member of the silane group, with the preferred hydrophobic compound being dimethyldichlorosilane. This reagent is commercially available dissolved in a volatile solvent, and a slide may conveniently be treated with this reagent in this form in a sealed container: in this case only a very small quantity of the reagent is required.

- 110 The masking medium is conveniently removed by treatment with a suitable solvent, e.g. acetone in the case of varnish. Treatment in this way is particularly useful if acetone/methanol treatment is subsequently to be applied to samples, e.g. for fixing mammalian cells, as this ensures that no materials will remain on the slide which will dissolve the acetone.

- 115 The slide may be of any desired material, and conveniently comprises 1 to 1.2 mm thick soda-lime glass. The slide may further be of any desired size and shape, and may for instance be of conventional standard size with use with existing microscope stages. As a further possibility a number, e.g. 4, of conventionally sized slides may be adapted to be assembled together to produce a larger composite slide for use with a special